Report by the

National Center for Toxicological Research,

U.S. Food and Drug Administration

An Interagency Agreement

FDA / NCTR

An Interagency Agreement Advancing Public Health Decisions

…conducting comprehensive toxicological assessment on chemicals of high priority to the FDA through the National Toxicology Program

printed April 2008

Food and Drug Administration

The **Food and Drug Administration (FDA)** is responsible for protecting the public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation. The FDA is also responsible for advancing the public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health.

National Center for Toxicological Research

The mission of the **National Center for Toxicological Research (NCTR)** is to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA. This research is aimed at understanding critical biological events in the expression of toxicity, and at developing methods to improve assessment of human exposure, susceptibility, and risk.

National Institute of Environmental Health Sciences

NTP Organization

The **National Toxicology Program (NTP)** resides within the National Institute of Environmental Health Sciences (NIEHS). The three core agencies that serve as founding and leadership agencies are NIEHS, the Food & Drug Administration's National Center for Toxicological Research (FDA/NCTR), and the Center for Disease Control and Prevention's National Institute for Occupational Safety and Health (CDCP/NIOSH). The Director of NIEHS is the Director of the NTP, and an Associate Director directs the operations of the NTP along with permanent staff at NIEHS. The primary oversight of the NTP is provided by the NTP Executive Committee, comprised of the heads of participating DHHS agencies (ATSDR, FDA, NCI, NIEHS, NIH) as well as heads from non-DHHS agencies concerned with public health (CDCP/NIOSH, CPSC, DOD, EPA, OSHA). Primary scientific oversight is provided by the NTP Board of Scientific Counselors, which is composed of scientists from academia, industry, and private entities. The FDA/NCTR and CDCP/NIOSH appoint representatives to the Board of Scientific Counselors.

National Toxicology Program (NTP)

NTP Mission

The mission of the **NTP** is to evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology. This interagency program was created in 1978, and envisioned as a cooperative effort to coordinate and manage the Department of Health, Education, and Welfare (now Health and Human Services) efforts on understanding the hazardous nature of chemicals to which the US public was exposed. The NTP maintains an objective, science-based approach in dealing with critical issues in toxicology and is committed to using the best science available to prioritize, design, conduct, and interpret its studies. To that end, the NTP is continually evolving to remain at the cutting edge of scientific research and to develop and apply new technologies.

Interagency Agreement between FDA and NTP

In December 1992, the NIEHS and FDA established a formal interagency agreement (IAG). The FDA requires toxicological data in order to make science-based decisions regarding safety and risk. With products where the FDA has no legal authority to require the regulated community to provide toxicological data, the FDA would nominate the chemical to the NTP for toxicological testing. The IAG established a mechanism where the NTP would support toxicology studies on FDA-regulated agents that were nominated to the NTP, and conduct the studies at the NCTR.

The original IAG provided resources to support five FDA priority chemical/agent NTP nominations. In 1995, because of the success of the concept, the NIEHS agreed to an open-ended IAG (no time or dollar limit). In 1996, the NIEHS and FDA agreed to expand the IAG to include important collaborative range-finding and multi-generation studies on five putative endocrine disruptor chemicals of interest to both the FDA and NIEHS. Most recently, in 1998 the FDA and NIEHS agreed to develop a state-of-the-art phototoxicity research and testing laboratory at NCTR (now designated as the NTP Center for Phototoxicology).

IAG Goals and Objectives

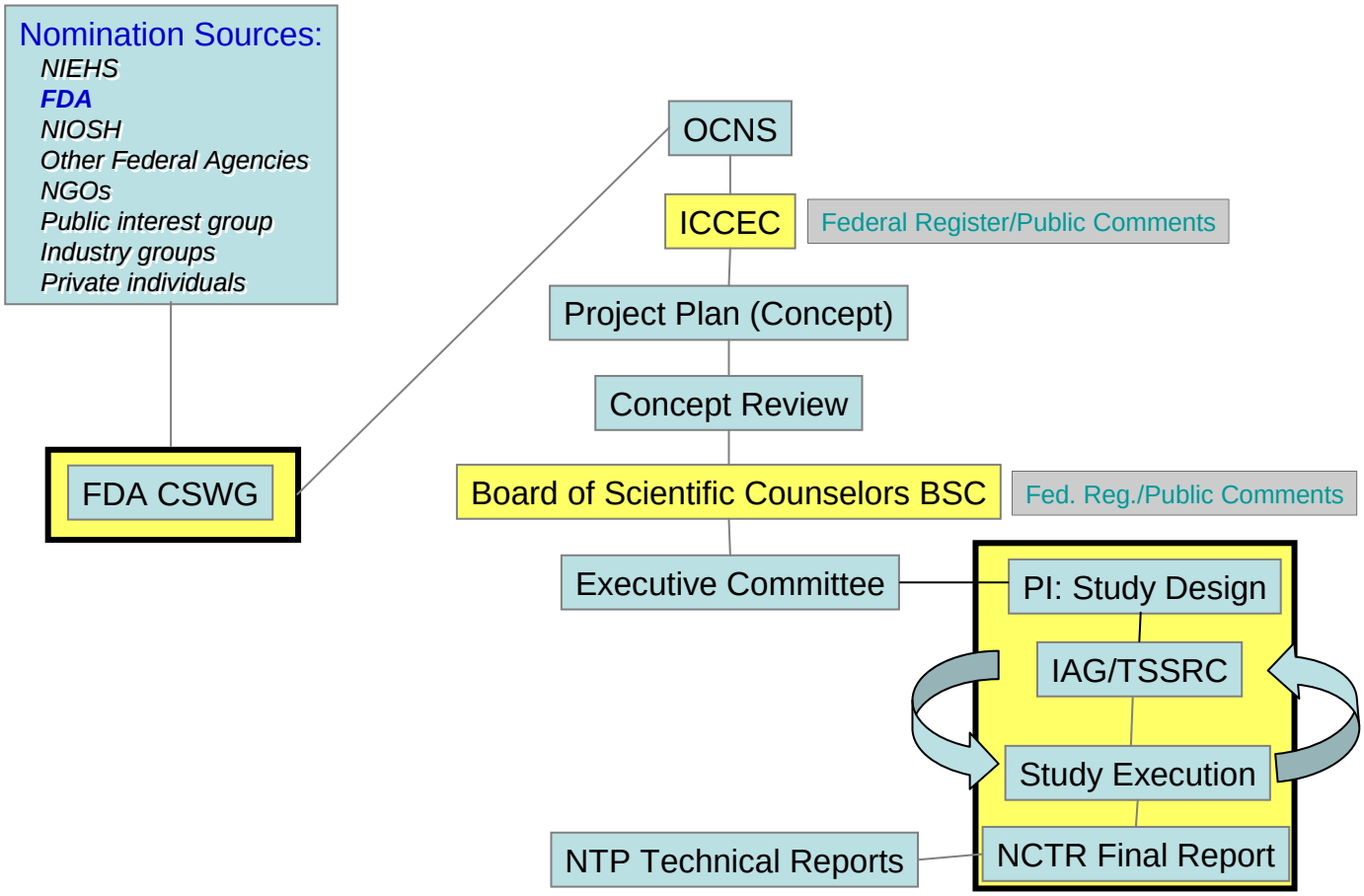
The primary goals of the IAG are to:

(1) Support the design and execution of toxicological studies that are consistent with the goals and needs of both the FDA and NTP;
(2) Provide oversight to ensure these studies are conducted in the most rigorous scientific manner;
(3) Ensure the data resulting from the studies are available to enable agencies to make science-based, safety assessment, and risk management decisions.

These goals of the IAG are met through the following objectives:

(1) Implementation of validated and, where appropriate, innovative, applied toxicological studies;
(2) Incorporation of appropriate mechanistic studies to assist in both study interpretation and safety and risk assessments;
(3) Provide information to the FDA regulatory and scientific community in a timely manner.

IAG Nomination Process



OCNS: Office of Chemical Nomination Selection

ICCEC: Interagency Chemical Coordination Evaluation Committee

CSWG: Chemical Selection Working Group

IAG: Interagency Agreement

TSSRC: Toxicology Study Selection & Review Committee

Organization of IAG

The conduct and progress of the studies under the IAG is monitored by the **Toxicology Study Selection and Review Committee (TSSRC)**. The committee is charged with:

(1) Reviewing the comprehensive research plans developed by the principal investigators (NCTR scientists);
(2) Recommending studies or modifications to studies that will enhance the regulatory utility of toxicity studies conducted on the IAG (e.g. carcinogenicity, reproductive/developmental, neurological, behavioral, immunological, mechanistic studies);
(3) Reviewing protocols developed by the principal investigators and making recommendations regarding the doses and study design;
(4) Considering and recommending alternative test systems (e.g. new born mouse assay, transgenic mice);
(5) Monitoring the progress of all studies conducted under the IAG.

Members of the TSSRC include the NCTR/FDA NTP Liaison, NCTR Director, Division of Biochemical Toxicology Director, NTP/NIEHS Project Officer, scientists from each of the other FDA Centers (especially those with regulatory responsibility for chemicals under study), NIEHS scientists, and scientists from academia with expertise on chemicals or methods under study. The TSSRC meets twice yearly and the recommendations of the TSSRC are forwarded to the Executive Team (NTP/NIEHS Project Officer and NCTR/FDA NTP liaison) for funding approval.

Research protocols are developed by NCTR scientists (principal investigators) to provide toxicology or mechanistic data identified in the nomination. Scientists from FDA or NIEHS may be co-investigators on the studies. These protocols are reviewed by NCTR TSSRC members, internally by NCTR scientists, and by other senior FDA scientists. Protocols recommended for funding are reviewed by NCTR support groups (e.g. Division of Biochemical Toxicology Chemistry Support Branch; Division of Microbiology Support Branch; Diet Preparation Support Contract; Animal Care Support Contract; Information Services Support Contract; Pathology Support Contract). Final approval of a protocol requires the signature of the principal scientist, supervisor, NTP/NIEHS project officer, NCTR/FDA NTP IAG liaison, and NCTR Center Director.

The products of these studies are technical reports or manuscripts (depending on the study) that are communicated with the appropriate FDA Center and Office, and then published in the public domain data to allow estimations of risk regarding the test article.

Studies Conducted Under IAG

The NTP Technical Report series is the primary venue for publication of the outcome of toxicology studies conducted under the IAG. Publication of scientific manuscripts in peer-reviewed journals is an additional measure of the completion of a project. To date, twelve Technical Reports have been completed and published on studies conducted under the IAG:

1. Fumonisin B_1 (TR-496)
2. Chloral Hydrate (TR-502)
3. Chloral Hydrate, Restricted Diet (TR-503)
4. Riddelliine (TR-508)
5. Urethane and Urethane combined with Ethanol (TR-510)
6. α-Hydroxy (glycolic) & β-Hydroxy (salicylic) Acids (TR-524)
7. Malachite Green (TR-527)
8. Genistein, multigeneration study (TR-539)
9. Genistein (TR-545)
10. Ethinyl Estradiol, multigeneration study (TR-547)
11. Ethinyl Estradiol (TR-548)
12. Aloe vera, topically applied (TR-553)

The studies of selected chemicals under the IAG have been conducted according to designs formulated to meet specific agency requirements outlined by the FDA Product Centers. Some of these studies have had common programmatic themes. Descriptions of the studies are presented in a programmatic outline as shown below.

FDA – NIEHS IAG
Chemicals / Agents Studied under the IAG

Multigeneration Studies Program, Endocrine Active Agents
- Methoxychlor
- Genistein
- Nonylphenol
- Vinclozolin
- Ethinyl Estradiol

Dietary Supplement Program
- Riddelliine
- Ephedra
- Bitter Orange/Citrus Aurantium
- Usnic Acid
- Aloe Vera, Oral
- Glucosamine/Chondroitin Sulfate

AIDS Therapeutics Program
- Combination of Zidovudine, Nevirapine, Lamivudine, Nelfinavir and Efavirenz
- Zidovudine, Nevirapine, Lamivudine in p53 Genetically Modified Mouse

Pediatric Program
- Ketamine
- Chloral hydrate
- Chloral hydrate, dietary restricted
- DEHP [Di (2-ethylhexyl) phthalate]

Phototoxicity Program
- Alpha & Beta Hydroxy Acids
- Aloe Vera, Topical
- Lemon & Lime Oil Furocoumarins
- Retinyl Palmitate
- Permanent Make-up Pigments

Nanoscale Materials Program
- Titanium Dioxide & Zinc Oxide
- Titanium Dioxide & Tg.Ac Transgenic Mouse
- Nanoscale Silver
- Nanoscale Gold

Food Contaminant Program
- Fumonisin B_1
- Urethane ± Ethanol
- Acrylamide
- Malachite Green
- Furan
- Melamine with Cyanuric Acid

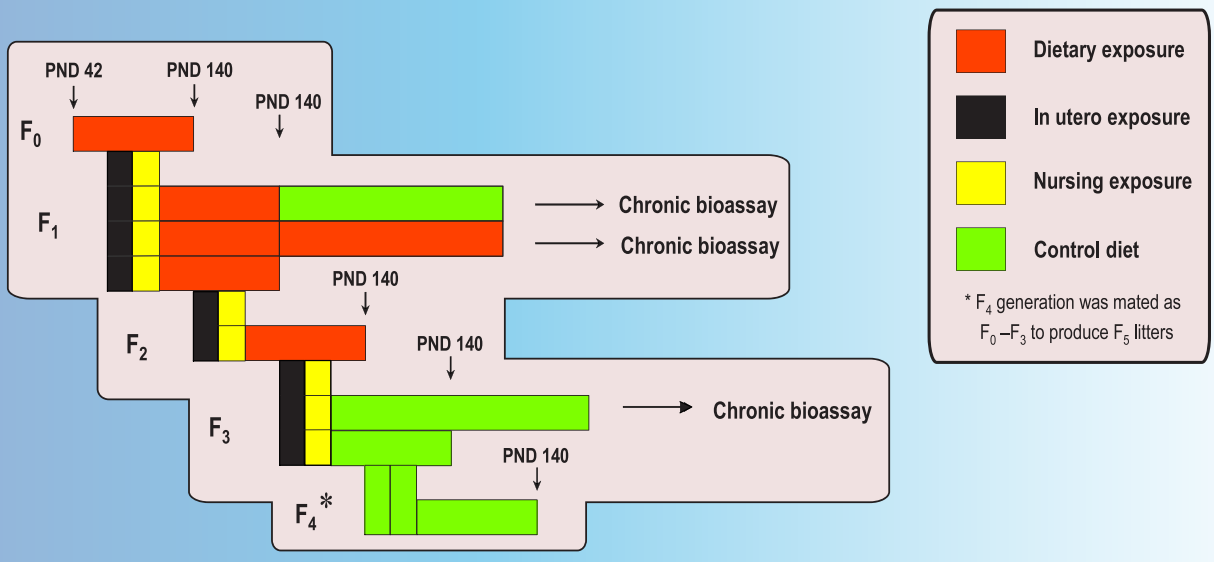
Multigeneration Studies Program on Endocrine Active Agents

At issue was that many compounds with low estrogenic activity are present in our environment. There was insufficient knowledge about the potential for estrogenic substances to cause adverse effects across generations.

The solution was to conduct studies on the potential long-term consequences of endocrine active agents administered at low to moderate doses over multiple generations.

Multigenerational studies are designed to test the Endocrine Disruptor Hypothesis, the hypothesis that exposure to a chemical during development or early in life will have consequences not only to the exposed entity, but also to subsequent generations. An additional hypothesis that follows is that adverse consequences are amplified with multiple generational exposures.

Multigeneration Dosing Schedule



Genistein

(Nominated by NIEHS, Supported by CFSAN)

• **Genistein** is an isoflavone that occurs in many types of natural foods. Human exposure to genistein occurs primarily through the consumption of soy-based food products, soy infant formula, and soy dietary supplements. Genistein is known to have weak estrogenic properties, as do many other isoflavones. Genistein was selected as one of the chemicals to examine in the extended multigenerational study due to its demonstrated estrogenic activity, and because the FDA regulates soy-based food products and dietary supplements.

• **Results**

In the multigeneration reproductive study with Sprague-Dawley rats, genistein at the highest dietary concentration in the study (500 ppm) reduced body weight gains, accelerated the timing of vaginal opening, slightly decreased anogenital distance, and altered estrous cyclicity in females across generations that continuously ingested genistein. There was some evidence for reduced litter size at 500 ppm in generations continuously exposed to genistein. Hyperplasia of the male mammary glands and calcification of renal tubules were observed at 100 and 500 ppm in continuously dosed male rats examined at 20 weeks of age. Female rats had an increase of pituitary and mammary gland adenomas/adenocarcinomas when examined after 2 years of continuous consumption of genistein at the highest dose level. Dietary genistein at 500 ppm also accelerated the onset of aberrant estrous cycles in female rats whether exposures were continuous or truncated at post natal day (PND) 140 or at weaning. Effects in male rats were minimal under the conditions of this study. The genistein technical reports have been peer-reviewed and published (TR539 and TR545).

Ethinyl estradiol

(Nominated by NCTR/FDA, supported by CDER)

- **Ethinyl estradiol** is a potent estrogen used in several therapeutics regulated by FDA, and is known to produce multiple structural and functional changes in animal models and humans at various levels of exposure. Because ethinyl estradiol is a potent estrogen and a prescription drug regulated by FDA, it was selected for toxicological testing.

- **Results**

  The effects observed from exposure to ethinyl estradiol at doses 2,500- to 10,000-fold lower than the genistein doses tested were generally similar to those reported in the genistein study, with effects on the onset of female puberty, altered estrous cycles, and hyperplasia of the male mammary gland being prominent. There was no marked indication of magnification of effects across exposed generations or carryover into unexposed generations (i.e. imprinted effects), critical aspects of the hypothesis being evaluated in these studies. The sensitivity of the male mammary gland as an indicator of effect, while not definitively an adverse effect, will be considered for inclusion in future testing protocols for endocrine disruptors. Ethinyl estradiol at the doses tested induced nonneoplastic and neoplastic uterine lesions after 2 year exposures.

Nonylphenol

(Nominated by NIEHS)

- **Nonylphenol (NP)**, is a commercial product used in the production of non-ionic surfactants following reaction with ethylene oxide. The resulting NP ethoxylates, which are the second largest group of non-ionic surfactants in commercial use, have a variety of industrial and consumer applications, including latex coatings and adhesives, paper and pulp production, textile manufacture and dyeing, agricultural chemicals, institutional cleaners, cosmetics, and spermicide. NP became a focus of attention as a potentially deleterious environmental estrogen only recently when it was reported that NP could be extracted from certain formulations of polystyrene and stimulate the growth and the induction of progesterone receptors in the estrogen-dependent cell line, MCF-7.

- **Results**

  The study expanded the data indicating the renal toxicity of Nonylphenol but did not support the reproductive toxicity of Nonylphenol under these study conditions. The final report is in preparation.

Vinclozolin

(Nominated by NIEHS)

Photo by Lynn Betts, USDA Natural Resources Conservation Service

Photo by Bob Nichols, USDA Natural Resources Conservation Service

• **Vinclozolin** is a dicarboximide fungicide that has a variety of agricultural and horticultural applications. This class of fungicides was designed to inhibit the synthesis of ergosterol, a major fungal steroid. Interest in the toxicological properties of vinclozolin has increased in recent years following a manufacturer-sponsored study that indicated significant developmental and reproductive toxicity. The toxicology of vinclozolin indicates that this chemical can induce a spectrum of effects consistent with that of an anti-androgen, and *in vitro* and *in vivo* mechanistic studies support this as the primary mechanism of toxicity. This compound was thus included as an anti-androgen in the IAG endocrine disrupter studies.

• **Results**

Upon review of the range finding results on vinclozolin, the TSSRC determined that further studies with this agent, while valuable, would not contribute to the overall understanding of the collected results for the estrogenic chemicals in the multigeneration/chronic studies of the endocrine disruptor program. Work on this compound did not proceed.

Methoxychlor

(Nominated by NIEHS)

• **Methoxychlor**, an insecticide, has been implicated as a potential cause of behavioral and reproductive anomalies in wildlife, and as an occupational human reproductive hazard. Because methoxychlor is widely used as a pesticide, it was recommended under the IAG to evaluate the potential adverse effects of both synthetic and natural agents with the capability of altering endocrine pathways.

• **Results**

Upon review of the range finding results on methoxychlor, the TSSRC determined that the level of the responses was insufficient to include methoxychlor in further segments of the multigeneration/chronic study design of the endocrine disruptor program. Work on this compound did not proceed.

Dietary Supplement Program

The FDA regulates dietary supplements under the Dietary Supplement Health and Education Act of 1994 (DSHEA), which states that the dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe before it is marketed. Generally, manufacturers do not need to register their products with FDA nor get FDA approval before producing or selling dietary supplements. Manufacturers must make sure that product label information is truthful and not misleading. As a result of this law, it is incumbent on the FDA to provide toxicology data to result in removal of an unsafe dietary supplement from the market.

The FDA and other federal agencies have nominated specific dietary supplements to the NTP for toxicology testing. The data that is provided from these studies allows the FDA to determine the level of risk associated with human use, and make regulatory decisions accordingly.

Riddelliine

(Nominated by CFSAN)

• **Riddelliine** is a pyrrolizidine alkaloid present in plants that grow in the central and western United States. Livestock ingesting such plants succumb to the toxic effects of riddelliine. In addition, residues have been identified in meat, milk, honey, teas, herbs, and dietary supplements consumed by humans. Other similar pyrroizidine alkaloids are known rodent carcinogens. CFSAN nominated riddelliine for a complete toxicological assessment.

• **Results**

Studies at NCTR under the IAG focused on mechanisms of action, while carcinogenesis studies were conducted at NIEHS. The mechanism of toxicity/carcinogenicity of riddelliine was similar to other pyrrolizidine alkaloids that are known contaminants in dietary supplements, with a common reactive intermediate for all pyrrolizidine alkaloids. This information was used by CFSAN to establish a warning about the consumption of dietary supplements which contain such alkaloids (e.g. the dietary supplement Comfrey). Contaminant-safe dietary levels for the pyrrolizidine alkaloids were also established.

Ephedra

(Nominated by Office of Dietary Supplements, NIH; supported by CFSAN)

- **Ephedra**, consumed in dietary supplements, resulted in many adverse event reports to the FDA, which included a few deaths associate with exercise. Ephedra was nominated for special studies assessing basic physiological, cardiovascular, and developmental outcomes in the presence and absence of caffeine and exercise.

- **Results**

  Studies were not conducted due to the 2004 enforcement action by FDA against dietary supplements containing ephedra products. Further mechanistic studies at the NIEHS confirmed the enhanced cardiotoxicity of ephedra, or ephedrine in combination with caffeine, when compared to each agent alone.

Bitter Orange/ Citrus Aurantium

(Nominated by CFSAN)

• **Citrus aurantium (bitter orange)** extracts have been used as a replacement for ephedra in many dietary supplements, especially those that are used for weight loss. Extracts contain synephrine, octopamine, carotenoids, as well as N-methyltyramine. Synephrine is the predominant component of the extract and is chemically similar to ephedrine with similar pharmacologic effects (sympathomimetic amine). In addition, published reports suggest an increased risk for adverse developmental effects. CFSAN nominated bitter orange for special studies assessing basic physiological, cardiovascular, and developmental outcomes in the presence and absence of caffeine and exercise.

• **Results**

The developmental toxicity potential of synephrine and bitter orange extract with and without caffeine was investigated in rats with no developmental toxicity noted. There were also no differences in fetal weight or in the presence of visceral or skeletal malformations.

Additional studies examining physiological effects of synephrine and bitter orange are on-going. It is anticipated that this study will be completed in late 2008 or early 2009.

Usnea, Usnic Acid

(Nominated by CFSAN)

• **Usnic acid** is a secondary metabolite of many species of lichen, particularly those of the genus *Usnea*. Usnic acid is a complex polycyclic chemical with known antimicrobial properties. Usnic acid has been used typically in traditional medicines and crude drugs in countries outside the U.S. It is also found in numerous consumer products in the U.S., including creams, toothpaste, mouthwash, deodorants, sunscreen products, and more recently in dietary supplements marketed to achieve rapid weight loss. Adverse event reports regarding liver failure, chemical hepatitis and milder hepatotoxic effects following use of dietary supplements have been received by the FDA. Usnic acid and Usnea were nominated by FDA/CFSAN in 2004 for complete toxicological assessment.

• **Results**

Preliminary studies have shown hepatotoxicity at the higher doses. Usnea lichen preparations were more toxic than equivalent doses of pure usnic acid. Toxicokinetic and *in vitro* mechanistic studies are currently being performed. Preliminary data have been shared with CFSAN.

Aloe vera, Oral Exposures

(Nominated by NCI, supported by CFSAN)

• **Aloe vera** is incorporated into a large number of commercial skin care products, and has gained popularity as a dietary supplement. Aloe vera is the most popular herbal alternative used today and the number of consumers exposed both orally and dermally is extremely high. Components of the leaf (gel, latex, and aloin) are known to have biological as well as toxic properties that can result in proliferative cell responses. Because of the number of consumers exposed to Aloe vera and the uncertain toxicological effects of such exposures, the NCI nominated it for complete toxicological evaluation by the NTP. Because Aloe vera is a product under the regulatory authority of the FDA, the studies were conducted under the IAG at NCTR with input from CFSAN. The studies were designed to investigate the toxic and carcinogenic potential of Aloe vera when administered orally via the drinking water.

• **Results**

Aloe vera whole leaf extract administered in the drinking water of rats and mice for 13 weeks resulted in decreased gastrointestinal transit times and significant reductions in body weights of rats, but not mice. Goblet cell hyperplasia was observed in the intestine of both species. Chronic 2-year bioassays have been conducted and the data are being evaluated. An NTP technical report is expected in 2009.

Glucosamine, Chondroitin and Glucosamine/ Chondroitin Combination

(Nominated by NCI, supported by CFSAN)

• **Glucosamine, chondroitin**, and their combination, are dietary supplements that are taken orally by a large number of people in an attempt to reduce inflammation and pain associated with osteoarthritis. Despite the fact that the mechanism of action is not fully understood and that there is little indication that the drugs can reach injured tissues when consumed orally, numerous studies have reported some degree of efficacy. Because of the possibility that there could be toxicities associated with the use of these products, and in view of large number of consumers exposed, NCI nominated glucosamine, chondroitin, and the combination of the two for complete toxicological assessments. Since these dietary supplements are under the regulatory authority of the FDA, a decision was made to conduct studies under the IAG at NCTR.

• **Results**

Toxicity studies of glucosamine alone and glucosamine in combination with chondroitin sulfate have been initiated. These studies are focusing on the potential toxicity of these supplements in diabetic rats which could be more susceptible to the sclerotic effects of glucosamine and/or chondroitin sulfate. Preliminary data have been shared with CFSAN.

Acquired Immune Deficiency Therapeutics Program

Acquired immune deficiency syndrome (AIDS) is caused by retroviral infection by the human immunodeficiency virus (HIV). Antiviral therapy is used to control the propagation of the virus and spread of the disease in affected individuals, and is used prophylactically in the treatment of newborns to AIDS-bearing pregnant mothers. The long-term health risks of AIDS therapeutics given peripartum to prevent AIDS transmission to newborns are currently unknown. The cancer risk of combinations of antivirals had not been examined in animal models at the initiation of the research program.

The goal of these studies is to understand the risk of peripartum treatment of newborns with antiviral compounds. The studies take advantage of the NCTR animal program, where it was demonstrated that newborn animals could be successfully dosed with test articles. Toxicity studies would include the use of AIDS therapies in animal models to test long-term risk from antiviral use, and investigate the use of transgenic animal models for rapid carcinogenicity screening.

AIDS Therapeutics

- Zidovudine
- Nevirapine
- Lamivudine
- Nelfinavir
- Efavirenz

(Nominated by NIEHS and NIH Office of AIDS Research, supported by CDER)

- Mother-to-child HIV transmission (vertical transmission) results in a significant number of babies born that are HIV positive. This transmission can be significantly reduced by treatment of the pregnant mother with one or more of the above anti-HIV drugs. Published studies in various animal models indicate that treatment during the last third of gestation results in increases of various tumors, especially lung, in their off-spring. Because data regarding the safety of anti-retroviral drugs administered during pregnancy are limited, NIH Office of AIDS Research and NIEHS asked NCTR to conduct special studies, with FDA oversight, funded through the IAG. Experimental designs include transplacental and transplacental/neonatal exposure for chronic bioassays and mutagenicity studies for each antiviral alone and in specific combinations used clinically.

- **Results**

  Two-year bioassays are being conducted to assess the effects of transplacental and transplacental/neonatal exposure of these anti-retroviral drugs and their combinations. In order to determine the mechanisms for the adverse effects of these drugs, other endpoints, including DNA incorporation, mutagenicity, and micronuclei induction are being measured. To date, data indicate that zidovudine combined with lamivudine increase genotoxicity when administered transplacentally and neonatally compared to transplacental treatment alone. Preliminary data have been shared with CDER and the NIH Office of AIDS Research.

AIDS Therapeutics (Zidovudine and Lamivudine) in Transgenic p53 Animals

(Nominated by NIEHS and NIH Office of AIDS Research, supported by CDER)

- Mother-to-child HIV transmission is a significant problem in the U.S. Such transmission is significantly reduced using a protocol that includes treatment of the pregnant mother with one or more antiviral therapeutics. This project tests the potential toxicity and carcinogenicity of perinatal and neonatal exposure of **zidovudine (AZT)** and **lamivudine (3TC)**, alone or in combination, with continuous and discontinuous dosing in a new transgenic mouse C3B6F1 trp53 (+/-). This new mouse model may reduce the time-to-toxic endpoint expression, thereby reducing cost and animal use.

- **Results**

  The dosing phase of subchronic studies with zidovudine has been completed and subsequent studies with zidovudine in combination with lamivudine and nevirapine are ongoing. Incidence of hepatocellular tumors were observed to be increased by zidovudine treatment. Preliminary data have been shared with both CDER and the NIH Office of AIDS Research.

Pediatric Program

The focal issue of this program is that many toxicological studies that support drug safety evaluations for use in adults are not required to evaluate the safety of the drug in a neonate or prepubescent animal model. Since many drugs are clinically used beyond the initial marketing intent, several drugs are being used in pediatric patients where no clear evaluation of age-dependent toxicity has been conducted. Several chemicals have been nominated to the NTP by the FDA to evaluate the safety of these drugs to newborn or adolescent animals, taking advantage of the non-human primate facility at NCTR.

Ketamine

(Nominated by CDER)

- **Ketamine** is a representative of a group of anesthetics which have been reported in the scientific literature to cause widespread and dose-dependent apoptotic neurodegeneration during brain growth in rodents after birth. Such anesthetics are used in pediatric medicine for procedures which often last several days or weeks. CDER nominated this drug to the NTP for the assessment of neurodegenerative changes using the non-human primate model with FDA and NICHHD funding. The NIEHS requested, and funded through the NTP, in vitro toxicity studies which resulted in the development of an effective mixed glial / neuronal cell culture system that has proven capable of mimicking the apoptotic responses seen in the brains of rodents and non-human primates.

- **Results**

  Ketamine-induced neural cell death in the developing rat cell culture model was largely apoptotic in nature; similar results have been seen in cultured non-human primate neural cells. Based on synaptogenesis, time of exposure becomes important when comparing rodent, non-human primate, and human neural cell toxicity outcome. Preliminary *in vivo* non-human primate studies demonstrate that measurement of apoptosis change is feasible and that cell death is likely both apoptotic and necrotic in nature; current studies are examining development-based exposure times and exposure duration to determine safe anesthetic exposure scenarios.

Chloral Hydrate

(Nominated by CDER)

• **Chloral hydrate**, a hypnotic used in pediatric medicine, was widely reported in the scientific literature to be genotoxic in several short term *in vitro* and *in vivo* tests. Numerous letters were written to the FDA requesting that the agency send warning letters to hospitals and clinics to stop using chloral hydrate during pediatric medical procedures. CDER nominated chloral hydrate to the NTP for complete toxicological assessment utilizing experimental designs unique to such assessments in the neonate (age of exposure and duration of exposure).

• **Results**

Female mice administered chloral hydrate were shown to have small increases in pituitary tumors (equivocal evidence of carcinogenicity) and no chloral hydrate induced tumors were seen in the liver of male mice treated as neonates. Mutagenicity (*in vitro* and *in vivo*) assays were inconclusive. CDER determined that the risk to pediatric patients undergoing medical procedures requiring the use of chloral hydrate was minimal and did not require additional labeling changes.

Chloral Hydrate, Dietary Controlled Animals

(Nominated by CDER)

- **Chloral hydrate** was nominated to the NTP by CDER for a complete toxicological assessment utilizing experimental designs in neonatal rodents to investigate the effects of diet and body weight on potential hepatocarcinogenicity in male B6C3F1 mice.

- **Results**

  Chloral hydrate administered by gavage to male mice for 2 years, was shown to be hepatocarcinogenic in both ad libitum-fed and dietary controlled mice. The latter mice were fed a restricted diet so that they gained weight at a pre-determined rate (i.e. dietary restriction). In these latter mice, the dose response of increased carcinogenicity was similar to the observed induction of liver enzymes that are biomarkers for peroxisome proliferation. Since peroxisome proliferators may not induce hepatocarcinogenicity in humans, CDER determined that the risk to pediatric patients undergoing medical procedures requiring the use of chloral hydrate was minimal and did not require additional labeling changes.

Di (2-ethylhexyl) phthalate (DEHP)

(Nominated by CDER and CDRH)

• **DEHP** is used as a plasticizer in medical devices such as blood, serum, dextrose, and saline bags, IV tubing, and dialysis machines. The potential toxicity (reproductive effects) of DEHP leaching from such medical devices to premature male infants has been identified as a concern by CBER and CDRH. While extensive literature on the biological effects of DEHP exists, much of this information deals with various adult rodent models; there are little data on the effects of DEHP in appropriate models for neonatal humans. CBER and CDRH suggested the neonatal non-human primate model should be used. These studies are designed to provide pharmacokinetic and biological data in the developing non-human primate.

• **Results**

Rodent studies have been completed for the development of mechanistic and analytical methods to be used in the non-human primate studies. The preliminary non-human primate pharmacokinetic studies have been completed. Protocol development is under way for a larger non-human primate study to focus on possible toxicity in the testes and the effects on Leydig and Sertoli cells. The data generated from these studies will provide CBER and CDRH important information for establishing safety guidelines for use of medical devices containing DEHP, especially when used in premature and neonatal infant care.

National Toxicology Program
Center for Phototoxicology

In the late 1990's it was recognized by the NTP and FDA that a research focus in the areas of phototoxicology and photocarcinogenesis was needed. Existing animal room and laboratory space was identified at NCTR for the location of a new research and testing facility. The space was renovated in 1998-1999 and designated as the National Toxicology Program Center for Phototoxicology.

The mission of the Center for Phototoxicology is to evaluate chemicals/agents of public health concern and to promote and protect human health by assessing the phototoxic and/or photocarcinogenic potential of these materials. Nominations for phototoxicology studies can include chemicals or drugs, topically or systemically applied, from the FDA, NTP, or other government agencies.

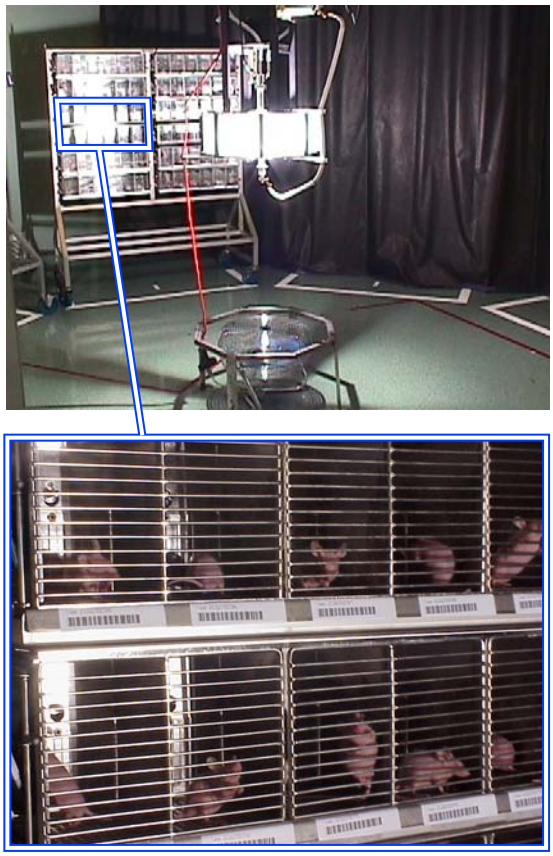
The uniqueness of the facility includes:

(1) The ability to dose animals with simulated solar light or other light sources (e.g. UVA, UVB, laser) that are of interest to regulatory agencies;

(2) State-of-the-art light dosimetry measurements;

(3) State-of-the-art animal imaging systems;

(4) Ability to conduct multiple, small- or large-scale animal studies, with complex study design in a GLP-compliant facility.



Alpha and Beta Hydroxy Acids

(Nominated by CFSAN)

• **Alpha and beta hydroxy acids** are included in a large percentage of cosmetic formulations where they are used as keratolytic agents to cause a restructuring of the skin and eliminate fine wrinkles (especially those induced by overexposure to sunlight). CFSAN nominated alpha and beta hydroxy acids to the NTP for phototoxicity and photocarcinogenicity testing due to concern that use of these products would result in increased susceptibility to sunlight-induced skin cancer. The studies were conducted in collaboration with CFSAN and photobiology experts (Argus Laboratories).

• **Results**

The results of the study indicate no increase in sunlight-induced skin cancer in mice when topically treated with an alpha hydroxy acid (glycolic acid). The treatment of the mice with a beta hydroxy acid (salicylic acid) resulted in a photoprotective effect from the sunlight induced skin cancers.

Aloe vera, Topical Application

(Nominated by NCI; Supported by CFSAN)

- **Aloe vera** is incorporated into a large number of commercial skin care products, and has gained popularity as a dietary supplement. Aloe vera is the most popular herbal alternative used today and a large number of consumers are exposed both orally and dermally. Components of the leaf (gel, latex, and aloin) are known to have biological as well as toxic properties that can result in proliferative cell responses. Because of the number of consumers exposed to Aloe vera and the uncertain toxicological effects of such exposures, the NCI nominated it for complete toxicological evaluation by the NTP. Because Aloe vera is a product under the regulatory authority of the FDA, it was decided that studies would be conducted under the IAG at NCTR with input from CFSAN. The studies were designed to investigate the potential phototoxic and photocarcinogenic properties of Aloe vera when dermally applied.

- **Results**

  Under the conditions of these studies, inclusion of aloe gel, aloe whole leaf, aloe decolorized whole leaf, and aloe-emodin in topical creams had a marginal effect upon the photocarcinogenesis of simulated solar light.

Lemon and Lime Oil Furocoumarins Including Oxypeucedanin

(Nominated by CFSAN)

- **Lemon and lime oils** are included in many FDA regulated products and in products that are available over the Internet. Although the regulated industry is aware of the phototoxicity of some furocoumarins (i.e., 8-methoxypsoralen), lime oil contains other furocoumarins. Oxypeucedanin is one of the most abundant furocoumarins in lime oil, and the concentration varies based upon the lime subspecies, the growth season and conditions, and processing. The studies were designed to determine if oxypeucedanin and other lime oil furocoumarins could be photoactivated to form DNA adducts, and to determine the relative phototoxicity of several furocoumarins including the psoralen, 5- and 8-methoxypsoralen, and oxypeucedanin.

- **Results**

  These studies would have provided CFSAN with data regarding the relative phototoxicities of naturally-occurring furocoumarins. After the start of the studies, CFSAN requested the studies be stopped, due to other requests of higher priority. The completed results indicated that purified oxypeucedanin can be photoactivated to form a DNA adduct *in vitro*, in a similar manner as 8-methoxypsoralen. A method for detecting oxypeucedanin and other furocoumarin DNA adducts was established.

Retinyl Palmitate

(Nominated by CFSAN)

- **Retinyl palmitate**, an ester of vitamin A, is employed in an increasing numbers of formulations for cosmetic use. Studies have shown that topical application of retinoic acid enhances skin tumor formation at lower doses of UV light than would typically induce sunburn, and the retinyl palmitate may be metabolized to retinoic acid in the skin. Retinyl palmitate was nominated for phototoxicity, photocarcinogenicity, and mechanistic studies based on the increasingly widespread use of retinyl palmitate in cosmetic products intended for use on sun-exposed skin and on the known biochemical and histological cutaneous alternations that occur with retinyl palmitate use, and to determine photodecomposition products that may be responsible for toxic outcomes.

- **Results**

  Mechanistic, phototoxicity, and photocarcinogenicity studies have been completed. Preliminary data that have been furnished to CFSAN indicate that retinyl palmitate is likely equivocal for photocarcinogenicity. Mechanistic study results have been published in the scientific literature and the technical report is currently being written. The technical report is projected for peer review and publication in 2009.

Permanent Makeup Pigments

(Nominated by CFSAN)

- **Permanent makeup** is the intradermal administration of pigments onto the face (e.g., eyeliner, lip color, lip liner) by a process identical to tattooing. Although there are no data on the number of women with permanent makeup, approximately 16% of the U.S. population has at least one tattoo. Adverse reactions to some permanent makeup inks were reported to the FDA starting in 2003, where women experienced severe reactions to permanent makeup inks. The manufacturer recalled the products and samples of the recalled material have been procured by the FDA. The nomination requested identification of the immunogenic material in the permanent makeup inks.

- **Results**

  These studies are in progress. A modification of the local lymph nodes assay is being used in these studies, where the insoluble inks are injected subcutaneously in the mice. The results to date indicate that recalled inks were immunostimulatory while permanent makeup inks from other sources were not immunostimulatory. Studies are in progress to isolate and identify the immunogen.

Nanoscale Material Program

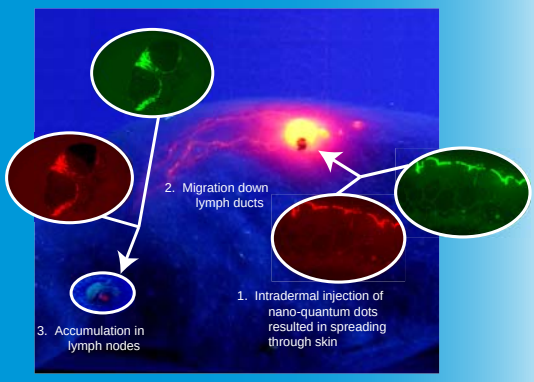
Nanoscale materials are typically defined as substances specifically synthesized with at least one dimension less than 100 nm (equals 0.1 micrometers); however, there is not universal acceptance of the 100 nm cutoff for upper size limit. The interest in nanoscale materials is due to unique properties that exist in some particles in the 10-100 nm size range that do not exist in larger dimension particles of the same chemical and physical makeup. Nanotechnology and nanoscale materials have been touted as the "next industrial revolution", resulting in considerable investment of industrial and government financial resources for development and marketing.

Nanoscale materials are being included in a variety of products in the U.S. market including cosmetics, human and animal drugs, devices and biologics either based on their inherent properties (e.g. bactericidal nano-silver, UV reflective nano-TiO2) or as a platform for transport of other molecules (e.g. fullerenes and dendrimers). There are few published studies detailing the safety of nanomaterials.

The studies in this program are part of the NTP Nanotechnology Safety Initiative, focusing on nanomaterials of regulatory interest to the FDA. The NTP has targeted several nanoscale materials for toxicological study including TiO2, fullerenes, single and multiwalled carbon nanotubes, and cerium oxide. The FDA is interested in understanding the toxicity of nanoscale materials that may be deliberately or inadvertently included in products the FDA regulates, which includes drugs, cosmetics, devices, food, dietary supplements, and food packaging.

Nanoscale Titanium Dioxide (TiO_2) and Zinc Oxide

(Nominated by CBEN, Rice University; Supported by CFSAN)



• Understanding the dermal penetration and distribution of topically applied nanoscale materials is one critical step in evaluating the toxicity of nanoscale materials in topical preparations such as sunscreen components. A collaborative study between CFSAN, NCTR, NTP, and Rice University was established to investigate the dermal penetration of (1) nanoscale fluorescent nanoscale semiconductor quantum dots as representative nanomaterials, and then (2) nanoscale TiO_2. The studies with quantum dots included skin penetration studies *in vivo* using mice (NCTR) and *in vitro* studies using human and pig skin (CFSAN). Additional studies were implemented to determine the dermal penetration of TiO_2 *in vivo* in mice (NCTR), determine the biological impact of dermal penetration with and without UV-containing light, and determine if TiO_2 penetrates minipig skin *in vivo* (later study under separate NCTR, CDER protocol).

• **Results**

The studies conducted under this program have used polyethylene glycol (PEG)-coated, nanoscale (23 nm), CdSe semiconductor quantum dots (QD) for distribution and skin penetration studies. Using intradermal injection as a means of introducing the QD into the skin, distribution of the QD was noted in the regional lymph nodes, liver and kidney. Topical application of the QD to mice did not result in skin penetration until the skin was damaged (dermabrasion). No penetration was found in human and pig skin *in vitro*. Similarly, skin penetration did not occur with TiO_2 *in vivo* in intact mouse or minipig skin. These results indicate that under normal application of nanoscale QD or TiO_2 in an oil-in-water emulsion, the materials do not penetrate intact skin.

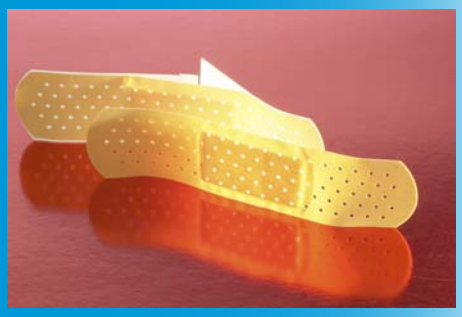
Nanoscale TiO_2 and Tg.AC Transgenic Mice

(Nominated by NIEHS, supported by CFSAN)

- **Nanoscale oxides of titanium** and **zinc** are used as physical sunblock agents in some sunscreens. TiO_2 exists primarily in two crystalline forms, rutile and anatase. Anatase TiO_2 is photochemically active, generating reactive oxygen species when irradiated with UV light. The haired Tg.AC mouse model, developed for the topical application of genotoxic and non-genotoxic toxicants with the production of skin tumors, will be used to investigate the application of nanoscale photoactive anatase TiO_2 along with irradiation with UVB/UVA light over a 20 week period. The animals will be monitored for tumor development .

Nanoscale Silver

(Nominated by FDA)



- **Nanoscale silver** was nominated by the FDA due to increased widespread use in drug, food and cosmetic products, and the general lack of data on the toxicology and pharmacokinetics of these materials. The potential for human exposure may be through manufacturing, use of home sanitizing kits, or use of consumer products containing nanoscale silver (e.g. clothing, textiles). In addition, intentional exposure may occur through ingestion of colloidal silver as a dietary supplement or use of the nanoscale products in wound dressings.

  Studies on the acute and chronic effects of nanoscale silver are currently being designed to address the existing data-gaps. These studies will be coordinated with other US regulatory agencies (CPSC, EPA) to ensure maximum use of the resulting data.

Nanoscale Gold

(Nominated by FDA)

• **Nanoscale gold** was nominated by the FDA based on increasing widespread use as a therapeutics platform, availability as a food supplement and the general lack of data on the toxicology and pharmacokinetics of nanoscale materials in general. Nanoscale gold is available commercially in both powder and colloid forms in various particle sizes. While there are several reports examining the effects of nanoscale gold *in vitro*, there have been few studies that have examined the effects of defined size and coatings *in vivo* in experimental animals. No long term *in vivo* pharmacokinetic and toxicity data of defined sizes and/or coatings exist in the public literature to assist in regulatory decisions.

The primary purpose of the research is to increase our basic understanding of how physiochemical properties impact the disposition, metabolism, elimination and toxicity of nanoscale gold. This project integrates with other studies being conducted as part of the NTP Nanotechnology Safety Initiative.

Food Contaminant Program

The FDA is responsible for assuring the safety of the food supply. This responsibility includes chemicals added to foods for preservation, flavor, color, or some other property. It also includes chemicals that contaminate the food supply either deliberately or accidentally through anthropomorphic activity, or due to biotic activity or contamination.

Fumonisin B_1

(Nominated by CFSAN, CVM, and USDA)

• **Fumonisin B_1 (FB_1)** is one of several toxic secondary metabolites produced by specific species of Fusarium genus of fungi. These fungi grow in many types of grain and food, and the species that produce FB_1 expand growth during drought. The toxicity of Fusarium toxins came to light when horses died after consuming grain contaminated with the Fusarium species. At the same time, reports from South Africa associated Fusarium species (and newly identified FB_1) with human esophageal cancer. Since humans are also exposed to FB_1 through contaminated grain crop foods, CFSAN and CVM, along with support from USDA, nominated fumonisins for a complete toxicological assessment. This was a collaborative effort between CFSAN, CVM, NCTR, USDA, NTP and Agriculture and Health Canada.

• **Results**

In mechanistic and carcinogenicity studies conducted at NCTR, it was determined that FB_1, the most abundant toxic metabolite, was the most toxic metabolite and clearly carcinogenic in rats and mice. It was further established that apoptosis and compensatory regeneration was the mechanism responsible for carcinogenicity. This study is considered the definitive study on FB_1 carcinogenicity, and the data were used by CFSAN, USDA, CVM, Agriculture Canada, Health Canada, and by FAO/WHO Joint Expert Committee on Food Additives (JECFA) for setting allowable contaminant levels in grains used in both animal and human food products.

Malachite Green

(Nominated by CVM)

• **Malachite green** is an antifungal triphenyl methane drug used to treat many fungal infections in veterinary medicine (and previously in human medicine). It is illegal to use in aquaculture within the U.S.; however, other countries have reported malachite green contamination of imported fish. As a result, CVM nominated this antifungal for a complete toxicological assessment.

• **Results**

Malachite green was found to cause liver tumors in rodents. CVM used these data in enforcement activities and in establishing residue hazard levels for unapproved animal drugs. The information was also used by the U.K. and Asian governments in establishing aquaculture residue hazard standards. This animal drug remains unapproved for use in aquaculture.

Urethane, combined with Ethanol

(Nominated by CFSAN)

• **Urethane**, a known rodent carcinogen, is the natural by-product of fermentation. There are particularly high concentrations of urethane in brandy and other types of dessert liquors and wines. In addition, there were no reliable urethane carcinogenic studies in the literature that could be used by the FDA to establish a risk assessment, and there was a concern that alcohol could potentiate the toxic effects of urethane. This combination of issues resulted in a nomination by CFSAN to the NTP.

• **Results**

Ethanol had a weak/mixed effect on the potentiation of urethane carcinogenicity. CFSAN has not yet conducted a risk assessment on urethane with or without ethanol. The bioassay data have been used by a recent FAO/WHO Joint Expert Committee on Food Additives (JECFA) review of urethane. The levels of urethane in distilled spirits have subsequently been reduced.

Acrylamide

(Nominated by CFSAN)

• **Acrylamide** is a known rodent carcinogen and a neurotoxicant. Recent studies have shown that acrylamide exists in high levels in baked and fried starchy foods (notably French fries, potato chips), bread, coffee, and other consumer food products. Acrylamide is formed as a natural by-product of the Maillard browning reaction (during cooking). Based on high levels of exposure to consumers through consumption of such products, and on preliminary risk assessments from existing but limited carcinogenicity studies in rodents, CFSAN nominated acrylamide for a complete toxicological assessment to include long-term carcinogenesis and neurotoxicity studies.

• **Results**

Mechanistic studies strongly supported a genotoxic mechanism of carcinogenicity based on the observations that acrylamide is mutagenic *in vivo* through metabolism to glycidamide, a reactive epoxide, which binds DNA and other macromolecules. A physiologically based pharmacokinetic model (PBPK/PD) has been designed and applied to extensive studies in male and female rats and mice. This same PBPK/PD model is being utilized to interpret limited literature data in humans which will improve subsequent risk assessments. The subchronic toxicity studies, Phase I and II neurotoxicology studies, and two year carcinogenicity studies have recently been completed. Upon analysis of the data, technical reports will be submitted to the FDA and the NTP for review.

Furan

(Nominated by CFSAN)

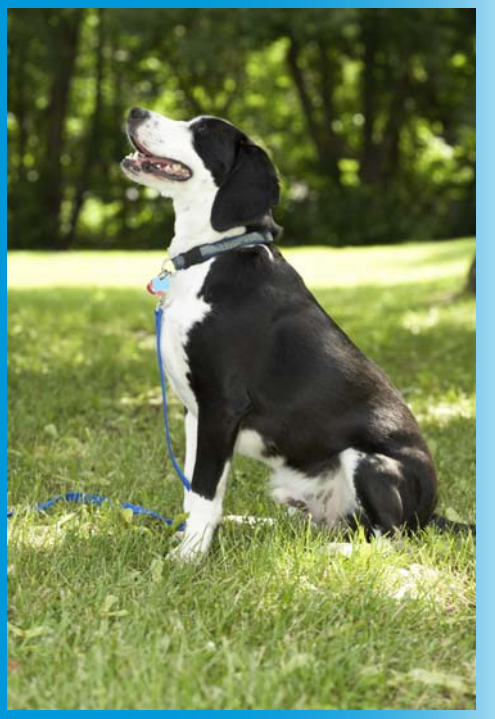
• **Furan** is formed in many common foods during heating of polyunsaturated fatty acids, carbohydrates or vitamin C. As a result, the most common sources of furan are canned and jarred foods such as baby foods, chili, coffee, savory snacks, and soups. The NTP previously determined that furan was carcinogenic in rats (bile duct cancer, mononuclear cell leukemia and liver tumors) and in mice (liver tumors). All doses used in these studies also caused cytotoxicity and necrosis in several organs. The FDA requested that studies be conducted at lower doses to examine the carcinogenicity of furan in the absence of these conditions.

• **Results**

Studies will be conducted in rats using doses of furan below those used in the previous NTP study. These studies will assess cell proliferation from short-term exposures, examine binding of furan metabolites to DNA, and evaluate carcinogenicity from lifetime exposure.

Melamine with Cyanuric Acid

(Nominated by CFSAN)



• **Melamine** is extensively used in industry in the preparation of polymers. An outbreak of sudden illness and renal failure-related death occurred in cats and dogs in the Spring of 2007. The FDA identified melamine and cyanuric acid as the contaminants in the adulterated pet food and responsible for the nephrotoxicity. A number of toxicity studies have been conducted with melamine and cyanuric acid demonstrating that these components individually have a low toxicity; however, recent data suggested that the exposure of mammals and fish to a combination of melamine and cyanuric acid resulted in crystal formation in the kidneys and nephrotoxicity.

• **Results**

Given the fact that combined melamine and cyanuric acid were toxic to mammals and fish, inadvertent exposure of humans to melamine with cyanuric acid could occur through consumption of adulterated food or contaminated meat or fish. Having no adequate exposure or toxicity data with mammals, the FDA nominated melamine with cyanuric acid to the NTP for in-depth toxicological characterization. The intent of the studies are to provide the FDA and other regulatory agencies with pharmacokinetic and toxicokinetic data to the extent that a no-observed-effect-level (NOEL) can be determined for mammals. This data would allow the FDA to refine its current risk analysis of melamine with cyanuric acid. Studies are expected to start in the summer/fall of 2008.

The NCTR vision for the 21st century is to be The Toxicology Research Center for the FDA, supporting the public health mission of the DHHS. NCTR is a state-of-the-art, Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited laboratory which contains modern scientific equipment, extensive animal research facilities, a comprehensive computational science infrastructure, and intellectual expertise in a broad array of scientific disciplines. NCTR will be recognized for its strong science and partnerships that effectively leverage resources through cooperative and/or collaborative agreements with other federal agencies, industry, and academia to assist in meeting the DHHS/FDA public health mission. The NCTR will continue to be a leader in FDA for mentoring a diversified pool of qualified scientists for academia, industry, and federal research regulatory agencies. As a national center, NCTR will provide technical training, educational opportunities, and growth potential for researchers through on-site training experiences.

The NTP vision for the 21st century is to support the evolution of toxicology from a predominantly observational science, at the level of disease-specific models, to a predominantly predictive science that is focused upon a broad inclusion of target-specific, mechanism-based, biological observations. The intent of the NTP vision is to expand the scientific basis for making public health decisions to include the potential toxicity of environmental agents.

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ATSDR	Agency for Toxic Substances and Disease Registry
CBEN	Center for Biological and Environmental Nanotechnology, Rice University
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CERHR	Center for the Evaluation of Risks to Human Reproduction
CFSAN	Center for Food Safety and Applied Nutrition
CPSC	Consumer Product Safety Commission
CVM	Center for Veterinary Medicine
DHHS	Department of Health and Human Services
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
ICCEC	Interagency Committee for Chemical Evaluation and Coordination
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
NCP	NTP Center for Phototoxicology
NCT	National Center for Toxicogenomics
NCTR	National Center for Toxicological Research
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NICHHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NSM	Nano Scale Material (<100 nanometers in size)
NIST	National Institute of Standards and Technology
NTP	National Toxicology Program
OOPD	Office of Orphan Products Development
OSHA	Occupational Safety and Health Administration
OWH	Office of Women's Health
ORA	Office of Regulatory Affairs
RoC	Report on Carcinogens
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
USDA	United States Department of Agriculture
UV	Ultraviolet

2008

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